

# Steam Volatile Constituents of Stale Nonfat Dry Milk. The Role of the Maillard Reaction in Staling

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In an effort to define chemically the stale flavor, 2-year-old skim milk powder was hydrated and extracted with dichloromethane. Steam volatile components were isolated by reduced-pressure low-temperature steam distillation of the extracted material, and fractionated by preparative gas chromatography on a Triton column. Individual fractions were analyzed on a 100-ft  $\times$  0.02-in. i.d. Carbowax 20M open tubular column, or on a 10-ft  $\times$  0.125-in. o.d. 10% Silicone SE-30 packed column, coupled to a mass spectrometer. Forty-four compounds were identified. Alkylpyrazines and pyrroles are

reported for the first time in nonfat dry milk, and furfuryl valerate, salicylaldehyde, methyl thio-benzoate, and dimethylsulfoxide are reported for the first time in a dairy product. Flavor thresholds and approximate concentrations were determined in an attempt to establish the importance of the volatile compounds from the Maillard browning in staling. Semiquantitative data suggest that 12 compounds, including 2-furaldehyde, 2-furfuryl butyrate, two alkylpyrazines, *N*-ethyl-2-formylpyrrole, *o*-cresol, benzaldehyde, and salicylaldehyde, may contribute to the stale flavor.

**F**lavor deterioration on storage is probably the main obstacle to a broader acceptance of skim milk powder and evaporated milk by the consumer. Aged or stale flavor is the single most important flavor criticism. Correlation between chemical identity of flavorful compounds and sensory detection has been established, to a large degree, for certain off-flavors such as oxidized, rancid, lactone, feed, metallic, etc. (Schwartz and Parks, 1965; Parks, 1967; Kinsella, 1969; Forss, 1969). On the other hand, as to the stale defect, it is admitted that it is a composite flavor, but no specific compound has been unequivocally associated with it, with the notable exception of *o*-aminoacetophenone (Parks *et al.*, 1964; Arnold *et al.*, 1966). However, on the basis of available evidence, it seems reasonable to propose that the stale flavor in dairy products is due to compounds arising from lipid deterioration and those resulting from the Maillard reaction (Henry *et al.*, 1948; Coulter *et al.*, 1951; Arnold *et al.*, 1966; Ramshaw and Dunstone, 1969a,b; Forss, 1969). Previous investigations by the authors on lactose-casein and whey model systems have provided information on volatile compounds which are likely to arise in dry skim milk during processing and storage, as a consequence of a Maillard-type deterioration (Ferretti *et al.*, 1970; Ferretti and Flanagan, 1971a,b). The present study was directed at the characterization of the steam volatile components of stale skim milk powder, with the hope of chemically defining the stale flavor and of determining the role, if any, of the Maillard reaction in staling. A satisfactory method of controlling staleness has not been discovered. The first step towards this end is the identification of the responsible chemical processes and compounds. The analytical methods developed in the study of the model systems were applied and the results are the subject of this paper. The starting material was a steam distillate obtained from a methylene chloride extract of stale skim milk powder. Gas-liquid chromatography (glc) and mass spectrometry (ms) were used to characterize the flavor compounds.

## EXPERIMENTAL

**Materials.** Twenty-eight-month-old instant nonfat dry milk was obtained from a well known manufacturer.

Authentic samples were either obtained from reliable commercial sources or synthesized by procedures described in the literature. Reference alkylpyrazines were gifts from the Corporate Research Department of the Coca-Cola Co., Atlanta, Ga. All compounds were purified by glc before determination of their spectral properties.

**Isolation of Vacuum Steam Volatile Oil.** The stale skim milk powder (4.45 kg) in batches of 400 g was suspended in dichloromethane, and deionized water (534 ml) was added dropwise during 30 min with vigorous mechanical stirring. The slurry was transferred into a Soxhlet thimble and extracted with 5.5 l. of  $\text{CH}_2\text{Cl}_2$  for 22 hr. On concentration to half volume and standing overnight at room temperature, 400 mg of crystalline urea precipitated. The bulk of dichloromethane was removed by careful distillation and the remainder with an aspirator. The fatty residue (primary extract, about 15 ml) was treated with 100 ml of water and steam distilled in a closed system at 5 mm pressure for 90 min. The bath temperature was held at 45 to 50°C, and the steam distillate was collected in two Dry Ice traps placed in series. The distillate (90 ml) was saturated with NaCl and extracted eight times with  $\text{CH}_2\text{Cl}_2$ . After drying over  $\text{Na}_2\text{SO}_4$  the solvent was removed as usual. The oily residue (secondary extract), having a strong stale flavor, weighed 90 mg (20 ppm of the original powder) and was analyzed as described below.

**Preparative Gas-Liquid Chromatography.** The secondary extract was fractionated into 15 cuts by means of a 14.5-ft  $\times$  0.25-in. o.d. stainless steel column packed with 60-80 mesh Chromosorb W (AW/DMCS treated) coated with 15% Triton X305 and 2% phosphoric acid, placed in a 5750 Hewlett-Packard gas chromatograph, equipped with a thermal conductivity cell. Twenty microliters of sample were injected each time, and the effluent was collected in 150  $\times$  1 mm (i.d.) glass capillary tubes cooled with Dry Ice. After trapping, the capillaries were sealed and kept at -20°C.

**Combined glc-Mass Spectral Analysis.** Cuts no. 1 and 2 were analyzed on a 100-ft  $\times$  0.02-in. i.d. capillary column coated with Carbowax 20M. The products of cuts 3 through

Table I. Compounds Identified in the Vacuum Steam Volatile Oil of Stale Nonfat Dry Milk

Compound	Approximate relative amount <sup>a</sup>
1 2-Acetylfuran <sup>b,c,d</sup>	s
2 2-Furaldehyde <sup>b,c</sup>	m
3 2-Furfuryl butyrate <sup>c</sup>	m
4 2-Furfuryl valerate	s
5 2-Methylpyrazine <sup>b,d</sup>	s
6 2,5- or 2,6-Dimethylpyrazine <sup>b,d</sup>	m
7 2,3-Dimethylpyrazine <sup>b,d</sup>	m
8 2-Methyl-5-ethyl- or 2-methyl-6-ethylpyrazine <sup>b,d</sup>	s
9 2,3,5-Trimethylpyrazine <sup>b,d</sup>	s
10 2-Formylpyrrole <sup>b,d</sup>	s
11 N-Methyl-2-formylpyrrole <sup>b,c,d</sup>	s
12 N-Ethyl-2-formylpyrrole <sup>c</sup>	s
13 2-Acetylpyrrole <sup>b,c</sup>	s
14 Maltol <sup>b,c,d</sup>	m
15 3-Hydroxy-2-butanone <sup>b,c,d</sup>	m
16 Acetol acetate <sup>b,c</sup>	m
17 Acetic acid <sup>b,c</sup>	m
18 Propionic acid <sup>b,c,d</sup>	l
19 Phenol <sup>b,c,d</sup>	s
20 o-Cresol	s
21 p-Cresol <sup>c</sup>	s
22 Benzaldehyde <sup>b,c,d</sup>	m
23 Salicylaldehyde	s
24 Acetophenone <sup>b</sup>	s
25 α-Methyl-γ-butyrolactone <sup>d</sup>	m
26 Dodecane	s
27 Tridecane	m
28 Methylenebenzenes (two)	m
29 Cumene	s
30 Naphthalene	s
31 Methylenebiphenylene	s
32 p-Dichlorobenzene	m
33 Benzothiazole	s
34 Methyl thiobenzoate	s
35 β-Ionone	s
36 Dimethyl sulfoxide	s
37 Dimethyl sulfone <sup>c,d</sup>	l
38 2-Nonanone	m
39 Butyric acid <sup>d</sup>	l
40 Isobutyric acid	m
41 Hexanoic acid	l
42 Octanoic acid <sup>c</sup>	s
43 Benzoic acid <sup>d</sup>	m

<sup>a</sup> l = large, m = moderate, and s = small. <sup>b</sup> Compound also identified by the authors in a lactose-casein model system (Ferretti *et al.*, 1970; Ferretti and Flanagan, 1971a). <sup>c</sup> Identified also in heated whey powder (Ferretti and Flanagan, 1971b). <sup>d</sup> Compound found also in commercial spray-dried whey (Ferretti and Flanagan, 1971c).

15 were chromatographed on a 10-ft × 0.125-in. o.d. column packed with 100–200 mesh Chromosorb W (AW/DMCS treated) coated with 10% Silicone SE-30. Both columns, stainless steel, were placed in a 5750 Hewlett-Packard gas chromatograph and coupled with an LKB-9000 mass spectrometer by means of a silanized 1.6-mm (o.d.) stainless steel capillary tube maintained at 200°C with an electric resistance. Temperature programming and flow conditions were varied, depending on the properties of the fraction being analyzed. Makeup carrier gas (He) was necessary with the capillary column. Mass spectral parameters were: ionizing energy 70 eV; ion source pressure  $5 \times 10^{-6}$  mm; ion source temperature 290°C; accelerating voltage 3.5 kV; electron multiplier voltage 2.5 kV; exit slit 0.3 mm; separator temperature 235°C. The washing of a few cuts with dilute Na<sub>2</sub>CO<sub>3</sub> was occasionally necessary to remove long chain fatty acids that would otherwise have interfered with the interpretation of the mass spectra.

## RESULTS

The 44 compounds identified in the present investigation and their approximate relative concentrations are in Table I. They were identified by interpretation of their mass spectra, followed by comparison with the mass spectra of authentic samples determined on the same instrument. Further verification was by comparison of the retention times on the Triton column, and on the Silicone or the capillary Carbowax column. Table II shows the mass spectra of the authentic and unknown compounds identified for the first time in dairy products.

The isomers 2,5- and 2,6-dimethylpyrazine, as well as 2-methyl-5-ethyl- and 2-methyl-6-ethylpyrazine, are not distinguishable by mass spectrometry. The presence of 2-methyl-3-ethylpyrazine is also suspected, but the spectrum was not intense enough for positive identification. The presence of two isomeric methylethylbenzenes was demonstrated by the observation of their (identical) mass spectra in separate glc peaks. The position of substitution in methylnaphthalene is not verifiable by mass spectrometry.

A number of compounds could not be identified. These included a series of C<sub>4</sub>-alkyl-substituted benzenes, a possible methyl thioether (mol wt 126), and two chlorinated compounds.

## DISCUSSION

The first 18 compounds of Table I are all products of nonenzymatic browning and were previously found in lactose-casein (Ferretti *et al.*, 1970; Ferretti and Flanagan, 1971a) and/or whey (Ferretti and Flanagan, 1971b) browning systems, with the exception of 2-furfuryl valerate. The three phenols 19, 20, and 21, benzaldehyde, and salicylaldehyde might also have arisen from lactose degradation, although their formation is known to require more drastic thermal conditions (caramelization) than those likely to occur during dry milk manufacture (Hodge, 1967). Consequently, precursors other than carbohydrates cannot be ruled out for them. Ramshaw and Dunstone (1969b) recently reported the formation of benzaldehyde during reaction of phenylalanine/lactose mixtures under unspecified conditions.

Of the numerous furans found in the model systems, only four were detected in stale dry skim milk. They do not include furfuryl alcohol, which seems to have undergone complete esterification to furfuryl butyrate and furfuryl valerate. The alkylpyrazines and the pyrroles are reported for the first time in nonfat dry milk. All the pyrazines in Table I were found also in edible spray-dried whey (Ferretti and Flanagan, 1971c) and in the lactose-casein model system (Ferretti *et al.*, 1970; Ferretti and Flanagan, 1971a), but were not found in whey subjected to accelerated browning (Ferretti and Flanagan, 1971b). There is no readily demonstrable explanation for this apparent discrepancy.

Furfuryl valerate, salicylaldehyde, methyl thiobenzoate, and dimethylsulfoxide have not been previously reported as constituents of dairy products or in studies of model systems.

Butyric and hexanoic acids, previously known to be the major free fatty acids in the whole and skim milk powders (Kawanishi and Saito, 1966), are the two major constituents of our vacuum steam volatile oil. The origin of saturated hydrocarbons such as dodecane and tridecane in nonfat dry milk is uncertain, although Sick and Lindsay (1968) showed that similar compounds can form by heating of butter oil above 200°C. The aromatic hydrocarbons 28 through 31 and β-ionone probably originate from feed, the latter from a carotenoid precursor.

If, as previously postulated, dimethylsulfone is derived from dimethylsulfide through metabolic oxidation (Ferretti and Flanagan, 1971c), dimethylsulfoxide appears as the obvious intermediate. The origin of *p*-dichlorobenzene, benzothiazole, acetophenone, and methyl thiobenzoate is unknown. *o*-Aminoacetophenone, previously identified in stale dry milk (Parks *et al.*, 1964), and undoubtedly an important constituent of the stale principle, was not detected.

In contrast to the results obtained with lactose-casein (Ferretti *et al.*, 1970; Ferretti and Flanagan, 1971a) and whey (Ferretti and Flanagan, 1971b) nonenzymatic browning systems, only one lactone has been encountered in the present study. This class of compounds is probably associated with relatively high temperature treatment of a browning mixture.

The gas-liquid chromatographic pattern (Triton X305 column and flame ionization detector) of a steam distillate, obtained as usual, from a commercial sample of dry skim milk purchased at a local supermarket, was qualitatively identical to that of the steam distillate obtained from the stale powder. Namely, each peak of one chromatogram matches an analogous peak in the other at the same retention time. Intensities, however, are different. The supposedly fresh powder was also found by the taste panel to be slightly stale. These observations convinced us that a complete glc-ms analysis of this sample was unlikely to provide qualitatively different results for useful comparison with those obtained with the stale powder.

**Flavor Significance of Stale Dry Milk Volatiles.** The flavor of all the compounds listed in Table I was evaluated by a panel of six expert judges who also estimated their flavor threshold in fresh skim milk. No single compound is associated with the typical stale flavor. The approximate concentration of each product was estimated by chromatographic peak area measurements. All the compounds are present in the powder in subthreshold amounts, assuming complete recovery. However, for 12 of them, the estimated ratio flavor threshold to concentration was lowest; therefore, they are likely to be more important in making up the stale flavor. They are: 2-furaldehyde, 2-furfuryl butyrate, methylethylpyrazine, 2,3,5-trimethylpyrazine, *N*-ethyl-2-formylpyrrole, *o*-cresol, benzaldehyde, salicylaldehyde, cumene, naphthalene, benzothiazole, and 2-nonanone.

A synthetic mixture, made up of all the identified compounds according to the estimated ratios, did not impart a stale flavor to fresh skim milk to which it was added at the 2-ppm level. Although the synthetic materials were all below threshold concentration, all judges but one indicated a response, ranging from grassy to medicinal, feed, beany, scorched, and foreign. This indicates that synergistic effects are operating.

That the substances responsible for the stale flavor were present in the vacuum steam volatile oil (secondary extract) was demonstrated by control experiments. Sixteen-hundred grams of stale skim milk powder was hydrated and extracted with dichloromethane, and the extract was processed exactly as described above. When the primary extract, or the steam distillate, or the secondary extract were reconstituted with fresh skim milk at the proper concentration, they all imparted the typical stale flavor present in the starting dry milk. Therefore, failure of the synthetic mixture to produce staleness might be due to the fact that the relative concentrations of the individual compounds are not sufficiently accurate, or to the absence in it of important constituents responsible for the stale flavor. Neither of these hypotheses is easy to verify. As previously mentioned, *o*-aminoacetophenone is

**Table II. Mass Spectra of New Compounds Identified in Stale Nonfat Dry Milk**

Methyl thiobenzoate, mol wt 152			Dimethylsulfoxide, mol wt 78		
Relative intensity			Relative intensity		
<i>m/e</i>	Authentic	Unknown	<i>m/e</i>	Authentic	Unknown
152	5	4	78	69	75
106	8	8	63	100	100
105	100	100	61	15	16
78	5	5	48	10	8
77	58	55	47	12	14
75	3	3	46	13	12
74	3	5	45	37	30
51	24	23	29	11	18
50	10	9	15	20	16

  

Furfuryl valerate, mol wt 182			Salicylaldehyde, mol wt 122		
Relative intensity			Relative intensity		
<i>m/e</i>	Authentic	Unknown	<i>m/e</i>	Authentic	Unknown
182	8	12	122	100	100
98	24	25	121	94	96
85	10	12	104	14	15
81	100	100	93	23	29
80	10	9	76	25	21
57	19	20	65	45	34
53	23	17	50	14	20
52	20	13			

an important compound in the stale flavor defect which was not found in our steam volatile oil. Its presence, however, can probably be taken for granted. When the synthetic mixture was added to fresh skim milk at the 2-ppm level with further addition of 2.5 ppb of *o*-aminoacetophenone, it was felt by three members of the panel that the resulting beverage exhibited a stale-type flavor similar to that of a 9% solution of the original stale commercial sample of nonfat dry milk, and more typically stale than a sample of fresh skim milk to which only *o*-aminoacetophenone (2.5 ppb) had been added.

## CONCLUSION

The results of this work support the view that the stale flavor in nonfat dry milk is due to a complex and balanced mixture of chemicals, rather than to a single compound. As to the role of the Maillard reaction, it is significant that at least five of the 12 constituents more likely to make a contribution to the stale flavor originate from nonenzymatic browning. Among them, 2-furaldehyde, and possibly benzaldehyde have also been implicated in the stale or gluey flavor of stored casein (Ramshaw and Dunstone, 1969b). A tentative conclusion from the above is that control of the Maillard reaction is likely to result in better keeping qualities of dry skim milk.

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